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Salt-stable association of simian virus 40 capsid with simian virus 40 DNA

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In 8 M CsCl, a fraction of the wild-type previrions and tsB228 nucleoprotein complexes lose their core histones but retain their capsid. These histone-depleted complexes apear in the electron microscope as a protein shell attached to supercoiled DNA. Consistent with this result, we find that in 1 M NaCl, the wild-type previrions dissociate into two populations of nucleoprotein complexes. One population sediments between 50 and 140 S and morphologically resembles the shell-DNA complexes isolated in CsCl gradients. The other population is comprised primarily of nucleoproteins which sediment at 40 S.

SV40 assembly *Protein–DNA interaction* *Nucleoprotein complex* *SV40 assembly mutant* *SV40 VP1* *Nonhistone protein*

1. INTRODUCTION

Simian virus 40 (SV40) DNA is packaged by a stepwise process in vivo. In the first step, the cellular core histones fold the SV40 DNA into nucleosomal units to form the viral chromatin or minichromosome [1]. The second step in SV40 DNA packaging involves the compaction of SV40 chromatin by the virus-coded capsid proteins VP1, VP2, and VP3 to yield the mature virion structure [1–12]. Little is known about the nature of the molecular interactions which trigger the second step in the assembly process or the possibility of a direct interaction between the capsid proteins and SV40 DNA. To uncover such interactions we have examined the dissociation products which result from exposing to high salt the wild-type SV40 previrions and the semiassembled particles formed in cells infected with the SV40 assembly mutant tsB228.

2. METHODS

All procedures used in this work have been described in [10–12].

3. RESULTS

3.1. *DNA-protein complexes isolated from tsB228-infected cells*

As observed for wild-type SV40 [2–12], under steady-state labeling conditions two major classes of viral DNA-protein complexes accumulate in tsB228-infected cells incubated at 33°C, the permissive temperature, or 37°C, semirestrictive temperature (fig.1A and B, respectively). In contrast, at the nonpermissive temperature (40°C), the block in virus assembly results in the accumulation of the 75 S chromatin and semiassembled virions which sediment between 100 and 160 S [10,12] (fig.1C). Biochemical and morphological studies have revealed that the 100–160 S complexes contain all 3 capsid proteins and appear as protein shells attached to minichromosomes in the electron microscope [12].

3.2. *Dissociation of the tsB228 semiassembled particles in CsCl*

Instability to high-salt is a distinctive characteristic of the intermediates in SV40 assembly. For example, immature virions and

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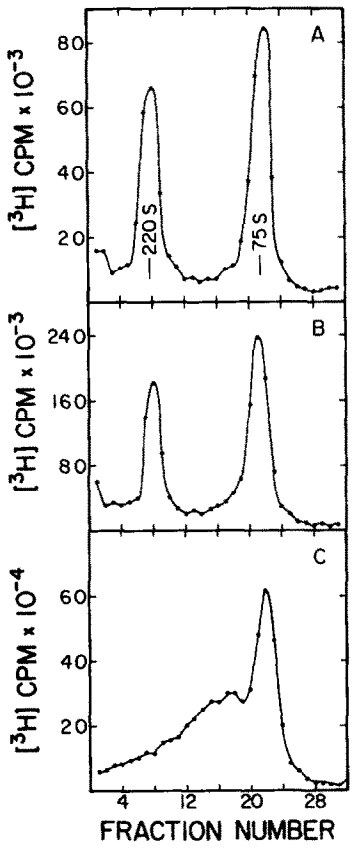

Fig.1. Sedimentation profiles of nucleoprotein complexes isolated from tsB228-infected cells. BSC-40 cells were infected with tsB228 and labeled at 32 h post-infection with [^{3}H]thymidine (100 Ci/150 mm dish) at (A) 33°C, (B)37°C, or (C) 40°C. The nucleoprotein complexes were isolated at 48 h post-infection from total cell lysate by Dounce homogenization and analyzed in sucrose gradients as described [10].

previrions dissociate in 8 M CsCl to yield primarily shells lacking DNA in addition to free DNA and proteins [2,4]. Our previous studies of another mutant of the B group (tsB201) have revealed that the tsB201 semiassembled particles also exhibit a similar sensitivity to high-salt [11]. When [^{3}H]leucine-labeled preparations of these particles were sedimented to equilibrium in CsCl gradients, the resulting ^{3}H pattern exhibited peaks at positions characteristic of dissociation products, as opposed to intact particles [12]. A major product banded at a density typical of shells lacking DNA, at ϱ = 1.30 g/cm^3, and minor dissociation products were observed to sediment as a rather broad peak encompassing densities unexpectedly higher than that of mature virions but lower than that of protein-depleted DNA [12]. The sedimentation behavior of the minor dissociation products suggested that some proteins, possibly a fraction of the semiassembled shells, must remain bound to DNA in the presence of CsCl.

To test this hypothesis, tsB228 semiassembled particle, labeled with [^{3}H]thymidine at 40°C as in fig.1C, were isolated and then sedimented to equilibrium in CsCl. The banding profile revealed 3 DNA-containing peaks: one at ϱ = 1.47 g/cm^3, corresponding to protein-depleted DNA and a doublet banding from ϱ = 1.36 to 1.42 g/cm^3 (fig.2B). This salt-stable material was further characterized by examining samples of each gra-

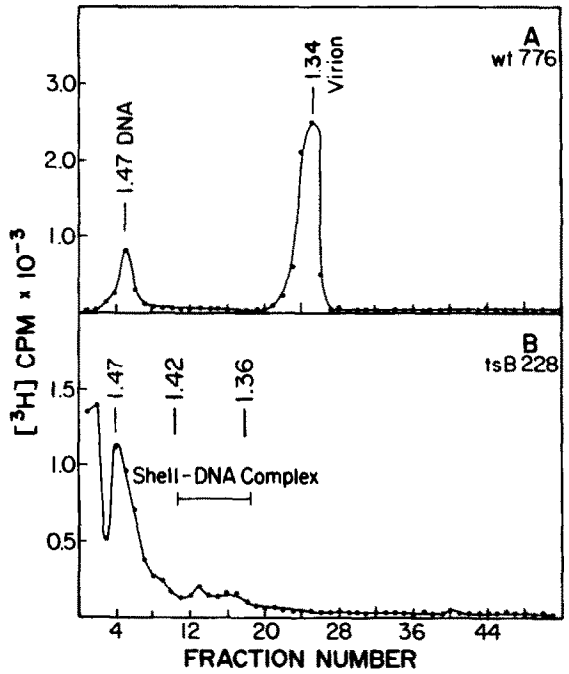

Fig.2. Stability of wt776 and tsB228 nucleoprotein complexes in CsCl. The infected cells were labeled with [^{3}H]thymidine at 40°C and the nucleoprotein complexes were isolated by centrifugation in sucrose gradients as described in fig.1. The fractions containing the wt virions and previrions, and tsB228 semiassembled particles (100–160 S) were layered separately in CsCl solutions and centrifuged to equilibrium. The banding patterns are shown in (A) for the wt virions and previrions, and (B) for the tsB228 semiassembled particles.

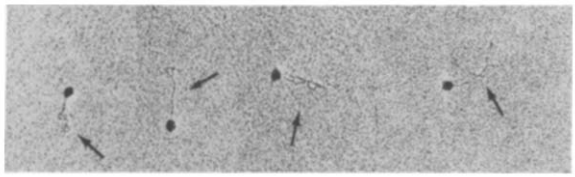

Fig.3. Morphology of tsB228 salt-stable nucleoprotein complexes. The material banding in CsCl gradients from ϱ = 1.36 to 1.42 g/cm^3, shown in fig.2B, was examined by electron microscopy. The arrows point to histone-depleted, supercoiled SV40 DNA attached to a shell-like structure.

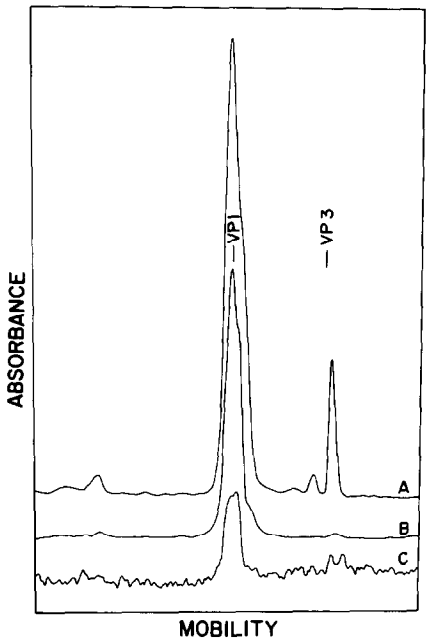

Fig.4. Protein composition of the dissociation products of tsB228 nucleoprotein complexes separated in CsCl gradients. Wild-type virions (220 S) and tsB228 nucleoprotein complexes (100–160 S) were obtained from infected cells labeled at 40°C with [^{3}H]leucine or [^{35}S]methionine, respectively, and then banded to equilibrium in CsCl as in fig.2. The peaks corresponding to wild-type mature SV40 (ϱ = 1.34 g/cm^3), tsB228 empty shells (ϱ = 1.30 g/cm^3), and tsB228 shell-DNA complexes (ϱ = 1.36–1.42 g/cm^3), were pooled, freed of CsCl by dialysis, and analyzed by SDS-polyacrylamide gel electrophoresis as described [12]. The tracings of the fluorogram of this gel show the protein composition of wt virions (A), tsB228 empty shells (B), and tsB228 shell-DNA complexes (C).

dient fraction by electron microscopy. We observed nucleoprotein complexes which appeared as a protein shell from which supercoiled DNA emanated (fig.3). SDS-polyacrylamide gel electrophoresis revealed a prominent protein band which comigrated with the major capsid protein VP1 (fig.4). We thus conclude that a fraction of tsB semiassembled particles remain stable in CsCl.

3.3. *Isolation of salt-stable shell-DNA complexes upon exposure of wild-type previrions to high-salt*

The isolation of salt-stable, shell-DNA complexes from tsB228 semiassembled particles prompted us to examine whether analogous complexes can also be obtained from cells infected with

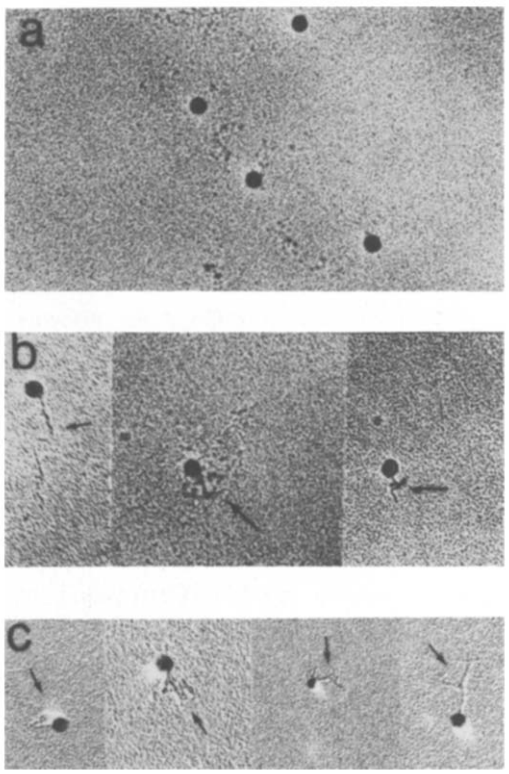

Fig.5. The morphology of wt previrion dissociation products in high-salt. The samples were prepared as in fig.2A and 6B and examined by electron microscopy. Panels A and B show the empty shells and shell-DNA complexes, which sediment in the 40–100 S region of the sucrose gradient, respectively. Panel C shows the shell-DNA complexes which band from ϱ = 1.36 to 1.42 g/cm^3 in CsCl gradients.

a wild-type SV40. For this study, a mixture of [^{3}H]thymidine-labeled previrions and virions – materials sedimenting from 200 to 210 S – were prepared from wt 776-infected cells and then banded to equilibrium in CsCl as above. In this case, we readily identified in the profile a DNA-containing complex at ϱ = 1.34 g/cm^3, corresponding to mature SV40, and a protein-depleted DNA peak at ϱ = 1.47 g/cm^3 (fig.2A). Unlike the profile obtained for tsB228, we could not discern DNA-containing peaks in the gradient region encompassing densities of 1.36–1.42 g/cm^3. However, when a sample of the gradient fractions was examined by electron microscopy, we detected complexes appearing as shell-like structures attached to histone-depleted DNA (fig.5C). This indicates that salt-stable shell-DNA complexes can also be obtained by exposing the wild-type assembly intermediates to high-salt.

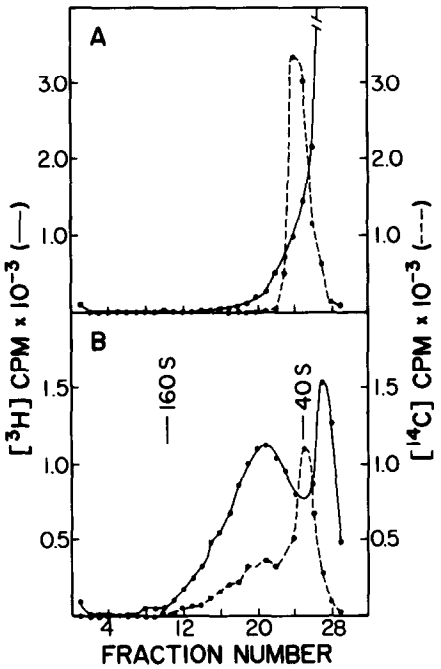

Fig.6. Stability of wt chromatin (A) and previrions (B) in 1 M NaCl. [^{14}C]Thymidine and [^{3}H]leucine-labeled chromatin and previrions were isolated from wt776-infected cells and diluted with a buffer containing 0.05 M Tris, pH 7.5, 2 M NaCl such that the final salt concentration was 1 M. The samples were subsequently sedimented on 15–31% sucrose gradients – made up in 1 M NaCl, 0.05 M Tris, pH 7.5 – and analyzed for proteins (——) and DNA (– – –).

It has been observed that in 1 M NaCl, the SV40 intermediates in assembly dissociate to yield shells lacking DNA and 30–40 S sedimenting DNA-protein complexes [4,7,11]. To extend the results described above, wild-type 180–200 S previrions and the 75 S chromatin, double-labeled with [^{3}H]leucine and [^{14}C]thymidine, were prepared, incubated in 1 M NaCl, and analyzed by sedimentation in sucrose gradients. While chromatin dissociated under these conditions to yield DNA-protein complexes sedimenting at about 40 S and free proteins (fig.6A), the previrions, on the other hand, dissociated to yield two populations of DNA-containing complexes: one at 40 S and another sedimenting from 50 to 140 S (fig.6B). Examination of a sample of the gradient fractions encompassing the 50–140 S region by electron microscopy revealed complexes consisting of shells attached to DNA (fig.5B) and empty shells (fig.5A).

4. DISCUSSION

Our findings agree with previously published results which showed that the interaction of the SV40 or polyoma major capsid protein VP1 with the viral DNA is unusually strong. For example, Etchison and Walter [13] have obtained an 80 S DNA-capsid complex by treating the polyoma virions with SDS. Griffith et al. [14] have isolated salt-resistant SV40 DNA-protein complexes by incubating the mature virions with a reducing agent under appropriate conditions. Finally, Brady et al. [15] have reported that the incubation of the SV40 virion-derived 110 S nucleoprotein cores [16–18] with sarkosyl results in the dissociation of VP2, VP3, and the core histones; VP1 remain attached to DNA in the form of a stable 30 S complex [15].

Proteins capable of salt-stable interactions with DNA may be considered to constitute a rare class all by itself [19–22]. This class includes the scaffolding proteins of the human metaphase chromosomes [19], the sequence-specific proteins which bind to the yeast centromeric DNA [20], and the nuclear matrix proteins [21]. The scaffolding proteins stabilize the HeLa metaphase chromosomes into highly organized structures even after the removal of the histones and most of the nonhistone proteins [22]. The isolation of salt-resistant shell-DNA complexes from the SV40 in-

termediates in assembly points to intriguing similarities between the capsid proteins of this virus and the scaffolding proteins of human chromosomes. For example, in addition to the salt-resistant DNA-binding property of these viral proteins, they, like the scaffolding proteins [22], also form a network of protein polymers mediated by divalent ions and disulfide bonds [16–19].

Currently, we could only speculate on the significance of the strong interactions between the SV40 capsid shell and the viral DNA. It is possible that such high affinity exhibited by VP1 for SV40 DNA may be involved in the initiation of virus assembly and/or the control of viral gene expression. Whatever its significance may be, in broader terms, it is becoming increasingly apparent that the papovavirus capsid proteins could serve as a useful model for investigating the interactions of nonhistone proteins with nucleo-histones and thus, the role of nonhistone proteins in organizing the structure of the more complex eukaryotic chromosomes.

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